SINGLE AND COMBINED EFFECTS OF *Meloidogyne incognita* AND *Fusarium oxysporum* f. sp. *lycopersici* ON TOMATO (*Solanum lycopersicum*) IN MAKURDI, NIGERIA

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SUMMARY

Most of the diseases caused by nematodes are debilitating. However, when they interact with other pathogenic organisms the disease scope is drastically altered. Simultaneous multiple disease infections are not uncommon in most tomato fields in Makurdi, Benue State. However, there is paucity of information on effects of concomitant infections on the agronomic performance of tomato involving *Meloidogyne incognita* and *Fusarium oxysporum* in the study area. This study was carried out to investigate the effects of nematode-fungus disease complex at different population densities under simulated micro plot conditions in Makurdi, Nigeria. Experiments were carried out in 2014 and 2015 at the Teaching and Research Farms of Federal University of Agriculture, Makurdi. Two weeks after planting (WAP), seedlings of tomato cv. Roma VF were inoculated with *Meloidogyne incognita* using 0, 2,500 or 5,000 eggs, solely and in combination with *Fusarium oxysporum* fungal inocula at three levels; 1,000, 10,000 or 100,000 conidia/ml by applying 5 ml of spore suspension per micro plot except in the control. Results showed that although single inoculation of either pathogens significantly ($P<0.05$) reduced plant growth, *M. incognita* reduced plant growth more. *F. oxysporum* inoculated at 1,000, 10,000 and 100,000 conidia/ml reduced plant height at 6 WAP by 14.2, 18.2 and 15.1 % in 2014 and by 15.2, 14.3 and 13.1 % in 2015, respectively. In the presence of the nematodes however, height of tomato plants at 6

WAP was 14.6 cm (2014) and 15.2 cm (2015) representing percentage reductions of 43.3 % and 35.8 %, respectively. The least fruit yields were observed in plants infected with combined inocula of *M. incognita* (5000 eggs) and *F. oxysporum* at 1000, 10,000 and 10,000 conidia/ml, representing 59.4, 61.5 and 65.8 % yield reduction, respectively in 2014, and 67.0, 67.8 and 73.3 %, respectively in 2015. The study concluded that the yield of Fusarium wilt-resistant tomato variety, cv. Roma VF was sub-optimal in the presence of the root-knot nematode. Further percentage reductions in its vegetative growth were also indicative of a possible synergistic activity targeted at the efficiency of the tomato host plant. Further studies on the dynamics of the population densities of the two pathogens at various intervals of the development of the host will further elucidate our understanding of the nematode-fungus disease complex.

Keywords: Disease complex, Fusarium wilt, Micro plots, Root-knot nematode, Tomato

TOMATO (*Solanum lycopersicum* L.) fruit constitutes one of the major condiments of diets used in most cuisines globally (3). The fruits are popular and frequently used for a number of delicious dishes such as stew, salad and sauce. They are also economical, shelf stable, and easy to use (20). It had been reported that consumption of tomato improves human health due to the high vitamin content of its fruit (24). Cultivation of tomato also serves as a source of income to farmers and other actors along the tomato value chain. In a study in Guyuk Local Government Area of Adamawa State, Nigeria, Zalkuwi *et al.* (28) showed that tomato production is profitable in both short and medium term owing to the positive gross margin and net income per hectare of N90,113.78 (≈ $237) and N68,296.26 (≈ $180), respectively. Despite the promising benefits of the crop, its production has been reported to be limited by the nematode – *Meloidogyne incognita (*Mi*)* and fungus – *Fusarium oxysporum* f.sp. *lycopersici (*Fol*)* among other diseases, hence keeping production at sub-optimal levels.

Most of the diseases caused by nematodes are debilitating (25). However, when they interact with other pathogenic organisms the disease picture is drastically altered. It changes from debilitating to

annihilating (21). Most often than not, even varieties bred for resistance to fungal attack become susceptible in the presence of nematodes (26). A vast majority of nematode interactions involve fungal pathogens, especially wilt and root-rot fungi. Of all the interactions of fungi with plant-parasitic nematodes, none is known to be more damaging to crops worldwide compared to the combined effects of wilt-inducing fungi and root-knot nematodes (4, 14).

Atkinson (2) for the first time, reported that infection by root-knot nematodes considerably increased incidence and severity of Fusarium wilt in cotton. Since then, a number of researchers have made efforts to work on the above subject. Choo *et al*. (6) studied the influence of *M. incognita* on the development of cucumber wilt by *F. oxysporum* f. sp. *cucumerinum*. They reported that wilt was much more severe in plants inoculated with nematode and fungus simultaneously than with the fungus alone. Plant growth was significantly reduced in plants inoculated with the fungus alone, nematode alone or both the pathogens simultaneously. Plant height and shoot weight were reduced more in plants inoculated with fungus and nematode simultaneously than by either pathogen alone. Root weight showed no statistical difference. The number of propagules of *F. oxysporum* f. sp. *cucumerinum* detected from the root and stem was higher in plants inoculated simultaneously with fungus and nematode than with fungus alone.

Kassab and Ali (12) studied the effect of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* on tomato cv. Walter, resistant to fungus. They observed that pre-inoculation with nematodes allowed fungus to readily and more extensively colonize the root than in plants inoculated with both the pathogens simultaneously or when the fungus preceded nematodes. Makhnotra and Khan (17) studied the interaction of *M. incognita* with *F. oxysporum* in rhizome rot of ginger under pot conditions. They reported that disease incidence was higher in plants inoculated with nematode and fungus simultaneously, than in plants inoculated with either pathogen singly. The nematode was found to be more damaging to plant growth than the fungus alone. Like in most climes, simultaneous multiple disease infections are not uncommon in most tomato fields in Makurdi, Benue State. However, there is paucity of information on effects of concomitant infections on the agronomic performance of tomato involving *Meloidogyne incognita* and *Fusarium oxysporum* in the study area.

The objectives of this study were to (i) examine the combined effects of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* on the yield response of tomato at different initial nematode population densities (IPD) in Makurdi, Benue State, Nigeria and (ii) underpin development of their disease complex through sequential analysis of plant growth and mortality under simulated micro plot condition.

MATERIALS AND METHODS

Isolation and maintenance of inocula

(a) *Meloidogyne incognita*

Three - month old *Meloidogyne*-infected *Celosia argentea* (source: National Horticultural Research Institute, Ibadan) plant roots were collected from six - litre experimental pots set-up in the screenhouse located at the Teaching and Research Farms of University of Agriculture, Makurdi (UAM). Hussey and Barker (10) method was followed for nematode egg extraction. The roots were washed to remove adhering soil particles and cut into 1- 2 cm segments. Pieces of roots were vigorously shaken in 0.5% sodium hypochlorite (NaOCl) solution for four minutes. The NaOCl solution was quickly passed through a 200-mesh sieve nested over a 500-mesh sieve to collect the freed eggs. The 500-mesh sieve with the eggs was then placed under a gentle stream of cold tap water to remove residual NaOCl. The egg suspension was thoroughly mixed using a magnetic stirrer. The number of eggs in 5 ml of suspension was counted in Doncaster counting dish under the dissecting microscope. An average of three counts was taken to estimate the egg population per ml of egg suspension. Nematode population density was adjusted to 2500 and 5000 eggs by inoculating tomato plants with 125 ml and 250 ml of concentrated nematode suspension. Distilled water (250 ml) was used as the control.

Motile stages of second-stage juveniles of *Meloidogyne incognita* were extracted from 5 g of symptomatic tomato roots and soil sample obtained from the experimental pots using modified Baermann tray technique (Whitehead) after 24 hours. Nematode specimens were identified based on morphological characteristics using Eisenback *et al.* (8) and Mai and Lyon pictorial keys (22).

(b) *Fusarium oxysporum* f. sp. *lycopersici*

Two - month old tomato plants (Roma VF) infected with *F. oxysporum* f. sp. *lycopersici* were collected from the Teaching and Research Farms of UAM and taken to the laboratory. *Fusarium oxysporum* f. sp. *lycopersici* was isolated from roots of infected tomato plants. Isolation of pathogen was carried out by cutting

the fragments at the border of diseased and healthy root tissue (16, 15). The infected plant materials were washed with tap water to remove the adhering soil. Small pieces of 1 to 2 mm size were cut from the juncture of diseased and healthy portion of roots with the help of a sterilized Razor® blade. These bits were surface-sterilized with 0.1M NaOCl for 10 to 20 seconds after which they were rinsed using distilled water under aseptic conditions. The bits were dried on sterilized filter paper to remove the excess moisture and subsequently, transferred to sterilized Petri dishes containing potato dextrose agar (PDA) medium.

The medium was supplemented with streptomycin (200 mgL^{-1}) after sterilization (autoclaved for 20 minutes) to prevent bacterial growth. The inoculated Petri plates were incubated at 27±1°C. Observations were made daily for fungal growth from tissue segments. When found, fungal mycelia were transferred onto fresh PDA until pure cultures of all isolated fungi were obtained. The emergent colonies were examined under microscope and identified with the aid of identification keys (19, 9). Fungal inoculum was maintained at 4°C for further studies on subsequently prepared PDA.

Establishment of field micro plots

Micro plot experiments were set up in 2014 and 2015 at the Teaching and Research Farms of Federal University of Agriculture, Makurdi, Nigeria. Each micro plot was made up of an open-ended plastic bucket (26 cm internal diameter x 26 cm in height), inserted to a depth of 20 cm and filled with 5 kg of heat-sterilized sandy loam soil. Two seeds of tomato cv. Roma VF per micro plot were directly planted. At two weeks after planting, seedlings were inoculated with *M. incognita* obtained from pure cultures at the rates of 0, 2500 and 5000 eggs, solely and in combination with fungal inocula at three levels; 1000, 10,000 and 100,000 conidia/ml by applying 5 ml of spore suspension per micro plot except, in the control and sole nematode-inoculated micro plots. A total of 12 treatments which included inoculation of seedlings using 2500 eggs of *M. incognita* ($M.i._{2500}$) alone, 5000 eggs of *M.i* ($M.i._{5000}$) alone, 1000 conidia/ml ($F.o._{1x10^3}$) of *F. oxysporum* alone, 10,000 conidia/ml ($F.o._{1x10^4}$) of *F. oxysporum* alone, 100,000 conidia/ml alone ($F.o._{1x10^5}$) of *F. oxysporum* alone, $M.i._{2500}$ + $F.o._{1x10^3}$, $M.i._{5000}$ + $F.o._{1x10^4}$, $M.i._{2500}$ + $F.o._{1x10^5}$, $M.i._{5000}$ + $F.o._{1x10^3}$, $M.i._{5000}$ + $F.o._{1x10^4}$, $M.i._{5000}$ + $F.o._{1x10^5}$ and control were evaluated in both years. The experiment was laid out in randomized complete block design (RCBD) and replicated three times on

an experimental plot size of 26 m x 8 m which accommodated 36 micro plots and an alley of 2 m, to avoid cross contamination.

Data collection and analysis

Data collected included plant height (cm) and number of leaves at three intervals namely; 3 weeks after planting (WAP), 6 WAP and 12 WAP, number of days to flowering, number of fruits per plant, fruit weight per plant (grammes) and fruit yield (kg/ha). Data were subjected to Analysis of Variance (ANOVA) using general linear model (GLM) procedure of GenStat 17th Edition (Lawes Agricultural Trust, VSN International, 2014) Statistical Software Package. Means were compared using Duncan's New Multiple Range Test at 5% level of probability. Grand means and coefficient of variation of each variate were calculated.

RESULTS

Effects of inoculation of Mi and Fol on growth and yield of tomato are presented in Tables 1 – 3. Although single inoculations of either pathogens resulted in reduced plant height, severe reductions were observed when both pathogens were present at 6 and 12 WAP. Fol at initial populations of 1000, 10,000 and 100,000 conidia/ml reduced plant height at 6 WAP by 14.2%, 18.2% and 15.1% in 2014 and by 15.2%, 14.3% and 13.1% in 2015, respectively (Table 1)

At the highest initial inocula levels for both pathogens average plant height of tomato at 6 WAP was 14.6 cm (2014) and 15.2 cm (2015) representing percentage reductions of 43.3% and 35.8%, respectively against the un-inoculated control plants. Similar reduction in plant growth was noticed at 12 WAP in micro plots containing nematode and fungus at higher initial population densities.

Single and combined inoculations of root-knot nematode and fungus had no significant effect ($p > 0.05$) on the number of leaves (NLs) at 3 WAP as shown in Table 2. At 6 and 12 WAP however, NLs were significantly affected by various combinations of the nematode and fungus. Lowest NLs were recorded when 5000 eggs of the nematode and 100,000 conida/ml of the fungus were inoculated resulting in 68.8% and 57.6% reduction in NL in 2014 and 2015, respectively. Similar trend was observed at 12 WAP.

Days to flowering (DAF) in plants inoculated with combined inocula of Mi and Fol was statistically longer ($p < 0.05$) compared to plants inoculated with either of the pathogens alone (Table 3). Tomato plants inoculated

with both *Fusarium oxysporum* and *M. incognita* took more than 50 days to flower except for plants inoculated with the lowest IPD of both pathogens.

Among all single inoculation treatments, *M. incognita* inoculated at 5000 eggs reduced number of fruits per plant the most (34.5% in 2014 and by 54.5% in 2015). On the other hand, highest IPD of *M. incognita* combined with individual three IPDs of *Fusarium oxysporum* (1000, 10,000 and 100,000 conidia/ml) had the greatest negative effect on NFP with a cumulative percentage reduction of 62.1%, 62.1% and 57.4%, respectively in 2014 and 73.2%, 73.2% and 76.8%, respectively in 2015 as against the un-inoculated control.

Fruits obtained from plants inoculated with individual pathogens weighed less compared to plants inoculated with both nematode and fungus except those singly inoculated with *M. incognita* (5000 eggs) and *Fusarium oxysporum* (10,0000 conidia/ml). *M. incognita* and *Fusarium oxysporum* at the highest IPD reduced weight of tomato fruit the most. The least average fruit weight (29.7 g) was observed in plants infected with combined inocula of *M. incognita* (5000 eggs) and *Fusarium oxysporum* (10,000 conidia/ml) in both 2014 (29.7g) and 2015 (25.3g) as shown in Table 3.

Furthermore, plants inoculated with combined IPDs of *M. incognita* and *Fusarium oxysporum* had lower fruit yield compared to those inoculated with single inoculations of either inoculum. The least fruit yields were observed in plants infected with combined inocula of *M. incognita* (5000 eggs) and *Fusarium oxysporum* at 1000, 10,000 and 10,000 conidia/ml, representing percentage in yield reduction of 59.4%, 61.5% and 65.8%, respectively in 2014 and 67.0%, 67.8% and 73.3%, respectively in 2015 (Table 3).

Table 1: Effect of *Meloidogyne incognita* and *Fusarium oxysporum* alone and in combination on plant height of tomato at three intervals in Makurdi, Nigeria

Treatment	Plant height (cm) at					
	3 WAP		6 WAP		12 WAP	
	2014	2015	2014	2015	2014	2015
Un-inoculated	8.1[a]	7.5[a]	25.3[a]	23.7[a]	60.7[a]	58.6[a]
M.i.$_{2500}$ alone	8.2(1.2)[a]	8.0(6.3)[a]	22.6 (10.7)[b]	24.1(1.6)[a]	55.2 (0.09)[b]	56.2(4.1)[b]
M.i.$_{5000}$ alone	7.9(2.5)[a]	7.2(4.0)[a]	20.2 (20.2)[cd]	22.6(4.6)[a]	50.4 (16.9)[c]	53.7(8.4)[bc]
F.o.$_{1x10}{}^{3}$ alone	7.7(4.9)[a]	8.0(6.3)[a]	21.7 (14.2)[bc]	20.1(15.2)[b]	53.3 (12.2)[bc]	56.1(4.3)[b]
F.o. $_{1x10}{}^{4}$ alone	8.2(1.2)[a]	7.3(2.7)[a]	20.7(18.2)[c]	20.3(14.3)[b]	51.4 (15.3)[c]	56.4(3.8)[b]
F.o. $_{1x10}{}^{5}$ alone	8.1(0.0)[a]	7.9(5.1)[a]	21.5(15.1)[bc]	20.6(13.1)[b]	53.0 (12.7)[bc]	55.1(5.9)[b]
M.i.$_{2500}$ +*F.o.*$_{1x10}{}^{3}$	8.0(1.2)[a]	7.6(1.3)[a]	20.2(20.2)[c]	21.6(8.9)[b]	50.5 (16.8)[c]	52.1(11.1)[bc]
M.i.$_{2500}$ + *F.o.* $_{1x10}{}^{4}$	7.9(2.5)[a]	8.2(8.5)[a]	18.6(26.5)[de]	18.3(22.8)[c]	47.2 (22.2)[d]	52.3(10.8)[bc]
M.i.$_{2500}$ +*F.o.* $_{1x10}{}^{5}$	7.2(11.1)[a]	8.2(8.5)[a]	17.9(29.2)[ef]	18.4(22.4)[c]	45.9 (24.4)[de]	53.6(8.5)[bc]
M.i.$_{5000}$ + *F.o.*$_{1x10}{}^{3}$	8.2(1.2)[a]	8.1(7.4)[a]	17.8 (29.6)[ef]	18.3(22.8)[c]	45.6 (24.9)[de]	50.2(14.3)[c]
M.i.$_{5000}$ +*F.o.* $_{1x10}{}^{4}$	8.0(1.2)[a]	8.0(6.3)[a]	16.5 (34.8)[f]	17.3(27.0)[cd]	42.9 (29.3)[e]	44.6(23.9)[d]
M.i.$_{5000}$ +*F.o.* $_{1x10}{}^{5}$	7.9(2.5)[a]	8.1(7.4)[a]	14.6 (42.3)[f]	15.2(35.8)[d]	38.3 (36.9)[f]	39.1(33.3)[e]
Grand Mean	8.0	7.8	19.8	20.0	49.5	52.3
[x]F pr. ($p \leq 0.05$)	0.268	0.74	0.02	1.03	0.05	0.01
[y]CV (%)	2.3	2.9	24.2	22.3	49.8	46.0

Means followed by the same letter in columns indicate no significant differences based on Duncan's New Multiple Range Test at 5 % level of probability; Each value is an average of three replications; Values in parentheses are percent reduction over un-inoculated control; ***M.i.*$_{2500}$** and ***M.i.*$_{5000}$** = Initial Population Densities of *Meloidogyne incognita* at 2500 and 5000 eggs/microplot; ***F.o.*$_{1x10}{}^{3}$**, ***F.o.*$_{1x10}{}^{4}$**and ***F.o.*$_{1x10}{}^{5}$**= Initial Population Densities of *Fusarium oxysporum* f. sp. *lycopersici* at $1x10^3$,$1x10^4$ and at $1x10^5$ conidia/ml; [x]**F pr. ($p \leq 0.05$)** = Fischer's probability value at 5% level of significance**;** [y]**CV** = Coefficient of Variation

Table 2: Effect of *Meloidogyne incognita* and *Fusarium oxysporum* alone and in combination on number of leaves of tomato in Makurdi, Nigeria

Treatment	Number of leaves per plant at					
	3 WAP		6 WAP		12 WAP	
	2014	2015	2014	2015	2014	2015
Un-inoculated	4.7[a]	4.1[a]	17.0[a]	15.8[a]	44.7[a]	42.5[a]
M.i.$_{2500}$ alone	4.3(8.5)[a]	4.0(2.4)[a]	12.7 (25.3)[b]	15.0(5.1)[a]	35.7 (20.1)[b]	40.4(4.9)[b]
M.i.$_{5000}$ alone	4.3(8.5)[a]	4.3(4.7)[a]	10.3 (39.4)[c]	13.5(14.6)[bc]	31.0 (30.7)[c]	38.4(9.6)[c]
F.o.$_{1x10^3}$ alone	4.3(8.5)[a]	4.1(0.0)[a]	11.7 (31.2)[bc]	14.3(9.5)[ab]	33.3 (25.5)[bc]	41.3(2.8)[bc]
F.o. $_{1x10^4}$ alone	4.3(8.5)[a]	4.1(0.0)[a]	11.3 (33.5)[bc]	13.5(14.6)[bc]	33.0 (26.2)[bc]	41.6(2.1)[bc]
F.o. $_{1x10^5}$ alone	4.3(8.5)[a]	4.3(4.7)[a]	10.3 (39.4)[c]	14.0(11.4)[ab]	31.0 (30.7)[c]	40.5(4.7)[bc]
M.i.$_{2500}$ +*F.o.*$_{1x10^3}$	4.7(0.0)[a]	4.1(0.0)[a]	10.3 (39.4)[c]	13.0(17.7)[c]	31.0 (30.7)[c]	38.1(10.4)[c]
M.i.$_{2500}$ + *F.o.*	4.3(8.5)[a]	4.1(0.0)[a]	9.7 (42.9)[cd]	10.2(35.4)[d]	29.7 (33.6)[d]	35.2(17.2)[d]
M.i.$_{2500}$ +*F.o.* $_{1x10^5}$	4.3(8.5)[a]	4.4(6.8)[a]	9.7 (42.9)[cd]	10.6(32.9)[d]	29.3 (34.5)[d]	35.2(17.2)[d]
M.i.$_{5000}$ + *F.o.*$_{1x10^3}$	4.7(0.0)[a]	4.2(2.4)[a]	8.7 (48.8)[d]	9.3(41.1)[de]	27.7 (38.0)[e]	29.2(31.3)[e]
M.i.$_{5000}$ +*F.o.* $_{1x10^4}$	4.3(8.5)[a]	4.1(0.0)[a]	7.7 (54.7)[d]	8.4(46.8)[e]	26.0 (41.8)[e]	27.1(36.2)[f]
M.i.$_{5000}$ +*F.o.* $_{1x10^5}$	4.7(0.0)[a]	4.3(4.7)[a]	5.3 (68.8)[e]	6.7(57.6)[f]	21.3 (52.3)[f]	19.3(54.6)[g]
Grand Mean	4.4	4.1	10.4	12.0	31.1	35.7
[x]F pr. ($p \leq 0.05$)	0.645	1.851	0.016	0.026	0.02	0.01
[y]CV (%)	1.6	1.0	23.6	24.1	47.2	59.3

Means followed by the same letter in columns indicate no significant differences based on Duncan's New Multiple Range Test at 5 % level of probability; Each value is an average of three replications; Values in parentheses are percent reduction over un-inoculated control; ***M.i.*$_{2500}$** and ***M.i.*$_{5000}$** = Initial Population Densities of *Meloidogyne incognita* at 2500 and 5000 eggs/microplot; ***F.o.*$_{1x10^3}$**, ***F.o.*$_{1x10^4}$**and ***F.o.*$_{1x10^5}$**= Initial Population Densities of *Fusarium oxysporum* f. sp. *lycopersici* at $1x10^3$,$1x10^4$ and at $1x10^5$ conidia/ml; [x]**F pr. ($p \leq 0.05$)** = Fischer's probability value at 5% level of significance**;** [y]**CV** = Coefficient of Variation

Table 3: Effect of *Meloidogyne incognita* and *Fusarium oxysporum* alone and in combination on yield of tomato in 2014 and 2015 in Makurdi, Nigeria

Inoculation	Number of Days to Flowering	Number of Fruits/plant	Fruit Weight/plant (g)	Fruit Yield (kg/ha)
	2014			
Un-inoculated	48.3[d]	8.7[a]	86.3[a]	16354[a]
M.i.$_{2500}$ alone	48.3[0.0][d]	7.7 (11.5)[b]	76.7 (11.2)[b]	14000 (14.4)[b]
M.i.$_{5000}$ alone	50.3 [4.1][c]	5.7 (34.5)[d]	66.0 (23.5)[c]	12906 (55.4)[c]
F.o.$_{1x10^3}$ alone	49.0 [1.4][cd]	7.7 (11.5)[b]	76.7 (11.1)[b]	13485 (17.5)[b]
F.o. $_{1x10^4}$ alone	49.0 [1.4][cd]	7.7 (11.5)[b]	76.6 (11.2)[b]	14551 (11.0)[b]
F.o. $_{1x10^5}$ alone	52.7 [9.1][b]	6.7 (22.9)[c]	66.5 (22.9)[c]	12580 (23.1)[c]
M.i.$_{2500}$ +*F.o.*$_{1x10^3}$	50.3 [4.1][c]	6.7 (22.9)[c]	66.7 (22.7)[c]	12580 (23.1)[c]
M.i.$_{2500}$ + *F.o.* $_{1x10^4}$	52.3 [8.3][b]	6.3 (27.6)[cd]	63.3 (26.7)[cd]	11951(26.9)[cd]
M.i.$_{2500}$ +*F.o.* $_{1x10^5}$	53.0 [9.7][ab]	5.7 (34.5)[d]	56.4 (34.6)[d]	10693 (34.6)[d]
M.i.$_{5000}$ + *F.o.*$_{1x10^3}$	53.7 [11.2][ab]	3.7 (57.4)[e]	35.2 (59.2)[ef]	6636 (59.4)[ef]
M.i.$_{5000}$ +*F.o.* $_{1x10^4}$	54.3 [12.4][a]	3.3 (62.1)[e]	33.3 (64.4)[ef]	6290 (61.5)[ef]
M.i.$_{5000}$ +*F.o.* $_{1x10^5}$	54.3 [12.4][a]	3.3 (62.1)[e]	29.7 (65.6)[f]	5598 (65.8)[f]
Grand Mean	51.3	6.1	58.9	11115
[x]F pr. ($p \leq 0.05$)	0.008	0.03	0.001	0.021
[y]CV (%)	1.8	8.5	7.3	7.3
[z]SE	0.74	0.42	3.49	659.6
2015				
Un-inoculated	46.3[d]	11.2[a]	93.8[a]	18222[a]
M.i.$_{2500}$ alone	47.3[2.2][d]	8.4[(25.0)[b]	73.9(21.2)[b]	13143(27.9)[b]
M.i.$_{5000}$ alone	49.6[7.1][c]	5.1(54.5)[d]	72.1(23.1)[c]	12571(31.0)[c]
F.o.$_{1x10^3}$ alone	50.1[8.2][cd]	7.3(34.8)[b]	72.5(22.7)[b]	13003(28.6)[b]
F.o. $_{1x10^4}$ alone	52.3[12.9][cd]	7.6(32.1)[b]	71.6(23.7)[b]	13415(26.4)[b]
F.o. $_{1x10^5}$ alone	52.5[13.4][b]	6.0(46.4)[c]	70.2(25.2)[c]	13611(25.3)[c]
M.i.$_{2500}$ +*F.o.*$_{1x10^3}$	49.9[7.8][c]	6.2(44.6)[c]	68.3(27.2)[c]	13100(28.1)[c]
M.i.$_{2500}$ + *F.o.* $_{1x10^4}$	51.7[11.7][b]	5.0(55.4)[cd]	67.5(28.0)[cd]	12900(29.2)[cd]
M.i.$_{2500}$ +*F.o.* $_{1x10^5}$	52.3[12.9][ab]	5.1(54.5)[d]	60.2(35.8)[d]	10500(42.4)[d]
M.i.$_{5000}$ + *F.o.*$_{1x10^3}$	51.5[11.2][ab]	3.0(73.2)[e]	37.1(0.6)[ef]	6006(67.0)[ef]
M.i.$_{5000}$ +*F.o.* $_{1x10^4}$	53.9[16.4][a]	3.0(73.2)[e]	33.0(64.8)[ef]	5859(67.8)[ef]
M.i.$_{5000}$ +*F.o.* $_{1x10^5}$	54.0[16.6][a]	2.6(76.8)[e]	25.3(73.0)[f]	4871(73.3)[f]
Grand Mean	50.9	5.9	62.3	11433.4
[x]F pr. ($p \leq 0.05$)	0.01	<0.001	<0.001	0.04
[y]CV (%)	0.05	0.43	0.34	0.34
[z]SE	0.69	0.72	6.05	1137.6

Means followed by the same letter in columns indicate no significant differences based on Duncan's New Multiple Range Test; Each value is an average of three replications; Values in parentheses ( ) are percent reduction over un-inoculated control while values in the square brackets [ ] are percentage increase against the un-inoculated control; ***M.i.*$_{2500}$**and ***M.i.*$_{5000}$**= Initial Population Densities of *Meloidogyne incognita* at 2500 and 5000 eggs/microplot; ***F.o.*$_{1x10^3}$**, ***F.o.*$_{1x10^4}$**and ***F.o.*$_{1x10^5}$**= Initial Population Densities of *Fusarium oxysporum* f. sp. *lycopersici* at $1x10^3$,$1x10^4$ and at $1x10^5$ conidia/ml; **F pr. ($p \leq 0.05$)** = Fischer's probability value at 5% level of significance**; CV** = Coefficient of Variation; **SE** = Standard Error.

Discussion

The present study showed that sub-optimal growth and yield characteristics were more pronounced when tomato plants were infected with root-knot nematode than in the absence of nematode infection. The presence of both pathogens resulted in reduced yield and vegetative growth as demonstrated by stunted growth, reduced number of leaves and sub-optimal yield. Relatively few studies have investigated the concomitant effects of such complexes on overall growth and yield of tomato. Roberts *et al*. (22) noted that in a cotton field infected with *M. incognita* and *Fusarium oxysporum* f. sp. *vasinfectum*, the slope of the regression model relating seed cotton yield to pre-plant nematode densities was greater than in a field infected only with *M. incognita*.

Similarly, Starr *et al.* (26) reported from micro plot experiments that the presence of *F. oxysporum* f. sp. *vasinfectum* had little effect on the threshold parameter of the Seinhorst model relating cotton yield to initial densities of *M. incognita*, but that the minimum yield parameter was lower in the presence of the wilt pathogen. Starr *et al*. (26) also reported that stunted growth was one of the most important consequences of the interaction of these two pathogens, and that interaction was most dramatic at intermediate population densities of both pathogens. At very high or very low densities of either pathogen there was no apparent interaction.

Findings of the current study corroborates report of Samuthiravalli and Sivakumar (23) who studied the interactive effect of *M. incognita* and *F. oxysporum* f sp. *lycopersici* on tomato cv. CO-3 under glasshouse conditions. They reported that the effect of nematode in combination with the fungus enhanced the suppression of plant growth than that of the fungus alone. Highest

reduction in plant height (33.08 cm) was observed in nematode fungus simultaneously inoculated plants.

There have been reports that root-knot nematodes provide entry sites for the fungus penetration through wounds produced during feeding (4, 1). Inoculation studies have shown that wilt was greater when nematodes were inoculated 2 to 4 weeks prior to the fungus, which indicates that wounding is not critical (21). Jenkins and Coursen (11) have shown that wilt severity was less from mechanical wounding than from wounds caused by the root-knot nematode, and some nematodes, despite their ability to feed on cotton, do not form a complex with the fungus (18).

Yield parameters of tomato evaluated during this present study were adversely affected when *M. incognita* was combined with *F. oxysporum* f. sp. *lycopersici*. These findings support earlier reports which indicated reduction in tomato yield as a result of combined inoculations of *F. oxysporum* with *M. incognita* (5). In a similar study by Kumar (13), fruit yield and other measured vegetative parameters were severely reduced in pots inoculated with *M. incognita* and *F. oxysporum* f. sp. *lycopersici*. The interaction between root-knot nematode and the fungus was more synergistic than antagonistic as evidently shown by the degree of stunting and yield loss observed in simulated complex. This could in part explain the reason for the comparatively high occurrence and severity of root-knot nematode-fungus wilt complex under field conditions previously reported based on the outcomes of preliminary survey conducted on some tomato fields in Benue, Kano, Ogun and Oyo States of Nigeria (7).

Conclusion

The study concludes that although tomato yield and growth were decreased individually by *M. incognita* and *Fusarium oxysporum* f. sp. *lycopersici*, percentage reductions increased when the two pathogens were both present indicating a possible synergistic activity targeted at reducing plant host efficiency. Further studies on the dynamics of the population densities of the two pathogens at various intervals of the development of the plant host will further elucidate understanding of the nematode-fungus disease complex.

REFERENCES

1. **Akram, M. and Khan, M.R. 2006.** Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* on tomato. *Annals of Plant Protection Sciences?* 14: 448-451.
2. **Atkinson, G.F. 1892**. Some Disease of Cotton. *Bulletin of. Alabama Agricultural Experimentation* 41: 61-65.
3. **Babalola, D.A., Makinde, Y.O., Omonona, B.T. and Oyekanmi, M.O. 2010.** Determinants of Post-harvest Losses to Tomato Production: A case study of Imeko–Afon local government area of Ogun State. *Journal of Life and Physical Science* (2): 14-18.
4. **Back, M.A., Haydock, P. P. J. and Jenkinson, P. 2002.** Disease Complexes involving Plant Parasitic Nematodes and Soil-borne Pathogens. *Plant Pathology* 51: 683-697.
5. **Bhagwati, B. and Goswami, B.K. 2000.** Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* on Tomato. *Indian Journal Nematology* 30: 93-94.
6. **Choo, H.Y., Lee, S.M., Kim, H.K. and Choi, Y.F. 1990**. Influence *of Meloidogyne Incognita* Infection on the Development of Cucumber Wilt by *Fusarium oxysporum* f. sp. *cucumerium. Korean Journal of Plant Pathology* 6: 412-416.
7. **Eche, C.O., Atungwu, J.J., Enikuomehin, O.A. and Ayodele, M. 2016.** Survey of Root- knot Nematode, Fusarium Wilt and Bacterial Wilt Disease Complexes in Tomato (*Solanum lycopersicum* L.) in Three Agro-Ecological Zones of Nigeria. *Nigerian Journal of Plant Protection*, 30 (1): 24-36.
8. **Eisenback, J.D., Hirschmann, H., Sasser, J.N. and Triantaphyllou, A.C. 1981.** *A Guide to the Four Most Common Species of Root-Knot nematodes (Meloidogyne spp.) with a Pictorial Key*. North Carolina State University Graphics: Raleigh, N.C. 79-105 pp.
9. **Gilman, J. C. 1957.** *A manual of Soil Fungi*. 2nd Ed. Ames, U.S.A.: Iowa State College Press. 392 pp.
10. **Hussey, R.S. and Barker K.R. 1973.** A Comparison of

Methods of Collecting Inocula for *Meloidogyne* spp. Including a New Technique. *Plant Disease reporter* 57: 1025-1028.

11. **Jenkins, W.R. and Coursen, B.W. 1957.** The Effect of Root-knot Nematodes, *Meloidogyne incognita*, *M. acrita* and *M. hapla*, on Fusarium Wilt of Tomato. *Plant Disease Reporter* 41: 182-186.
12. **Kassab, A.S. and Ali, M.K. 1995.** Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* on a Resistant Tomato Cultivar. *Annals of Agricultural Sciences* 40: 913-922.
13. **Kumar, V. 2008.** Studies on *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani* Disease Complex of *Lycopersicon esculentum* and its Management through Integrated Approach. Ph.D. Thesis, Aligarh Muslim University, India. 301pp.
14. **Luc, M., Sikora, R.A. and Bridge, J. 2002.** *Plant-parasitic Nematodes in Subtropical and Tropical Agriculture*.CAB International, Wallingford, UK. 629 pp.
15. **Machado, J.C., Langerak, C.J. and Jaccoud-Filho, D.S. 2002.** Seed-borne fungi: A Contribution to Routine Seed Health Analysis. International Seed Testing Association, Zürich, Switzerland.
16. **Mai, W. F. and Lyon, H. H. 1975.** Pictorial Key to General of Plant-parasitic nematodes. No.4th Ed. (revised) pp. 221. Not complete. What is the number, Pub
17. **Makhnotra, A.K. and Khan, L.M. 1997.** Assessment of Yield Losses in Ginger due to *Meloidogyne incognita. Indian Journal of Nematology* 11: 259-260.
18. **Manzanilla-López, R.H. and Starr, J.L. 2009.** Interactions with other Pathogens. In: Perry, R.N., Moens, M. and Starr, J.L. (Eds.), *Root-knot Nematodes*. CAB International, Wallingford, UK. 223-240 pp.
19. **Mathur, S.B. and Kongsdal, O. 2003.** Common Laboratory Seed Health Testing Methods for Detecting Fungi. International Seed Testing Association, Switzerland. 224 pp.
20. **Oyinlola, E.Y. and Jinadu, S.A. 2012.** Growth, Yield and Nutrient Concentrations of Tomato as Affected by Soil Textures and Nitrogen. *Asian*

Journal of Agricultural Research 6 (1): 39-45.

21. **Powell, N.T. 1979.** Internal Synergism among Organisms Inducing Disease. In: Horsfall, J.G. and Cowling, E.B. (Eds.), *Plant Disease* Vol. IV. Academic Press, New York, USA. 13-33 pp.
22. **Roberts, P.A., Matthews, W.C. and Ehlers, J.D. 1996.** New resistance to virulent root-knot nematodes linked to the Rk locus of cowpea. *Crop Science* 36: 889-894.
23. **Samuthiravalli, M. and Sivakumar, M. 2008.** Interaction of Meloidogyne incognita with *Fusarium oxysporum* f. sp. *lycopersici* on Tomato. *Annals of Plant Protection Sciences* 16: 182-184.
24. **SCANNERS, 2009.** Understanding the Health Benefits of Tomato Products. *Sports, Cardiovascular and Wellness Nutrition* 2(2): 1-4.
25. **Sikora, R.A. and Fernandez, E. 2005.** Nematode Parasites of Vegetables. In: Luc, M., Sikora, R.A. and Bridge, J. (Eds.), *Plant-parasitic Nematodes in Subtropical and Tropical Agriculture, 2nd edition.* CAB International, Wallingford, UK. 319-391 pp.
26. **Starr, J.L., Jeger, M.J., Martyn, R.D. and Schilling, K. 1989.** Effects of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *vasinfectum* on Plant Mortality and Yield of Cotton. *Phytopathology* 79: 640-646.
27. **Whitehead, A.G. and Hemming, J.R. 1965.** A Comparison of Some Quantitative Methods of Extracting Small Vermiform Nematodes from Soil. *Annals of Applied Biology* 55: 25-38.
28. **Zalkuwi, J., Maurice, D.C., Garba, A. and Usman, J. 2012.** Profitability of small-scale dry season tomato production in Guyuk local government area of Adamawa State, *Nigeria Journal of Technology and Education Research* 5(1): 78-82.